In vitro Antibacterial and Chemical Properties of Essential Oils Including Native Plants from Brazil against Pathogenic and Resistant Bacteria

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Abstract: The antimicrobials products from plants have increased in importance due to the therapeutic potential in the treatment of infectious diseases. Therefore, we aimed to examine the chemical characterisation (GC-MS) of essential oils (EO) from seven plants and measure antibacterial activities against bacterial strains isolated from clinical human specimens (methicillin-resistant Staphylococcus aureus (MRSA) and sensitive (MSSA), Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhimurium) and foods (Salmonella Enteritidis). Assays were performed using the minimal inhibitory concentration (MIC and MIC90%) (mg/mL) by agar dilution and time kill curve methods (log CFU/mL) to aiming synergism between EO. EO chemical analysis showed a predominance of terpenes and its derivatives. The highest antibacterial activities were with Cinnamomun zeylanicum (0.25 mg/mL on almost bacteria tested) and Caryophyllus aromaticus EO (2.40 mg/mL on Salmonella Enteritidis), and the lowest activity was with Eugenia uniflora (from 50.80 mg/mL against MSSA to 92.40 mg/mL against both Salmonella sources and P. aeruginosa) EO. The time kill curve assays revealed the occurrence of bactericide synergism in combinations of C. aromaticus and C. zeylanicum with Rosmarinus officinalis. Thus, the antibacterial activities of the EO were large and this can also be explained by complex chemical composition of the oils tested in this study and the synergistic effect of these EO, yet requires further investigation because these interactions between the various chemical compounds can increase or reduce (antagonism effect) the inhibitory effect of essential oils against bacterial strains.

Key words: GC-MS, antibacterial activity, terpenes, plant secondary metabolites, bacteria

1 INTRODUCTION

The emergence of bacterial resistance can occur by different mechanisms that are usually related to selective pressure and the inappropriate use of antimicrobial drugs¹ and there is a prevalence of multiresistant bacteria as penicillin-resistant Streptococcus pneumoniae, Escherichia coli and multiresistant Pseudomonas aeruginosa². The methicillin-resistant Staphylococcus aureus (MRSA) is a nosocomial pathogen that causes morbidity and mortality worldwide and options for the treatment of MRSA infections are limited³. Besides these, resistant strains of Salmonella spp. can be isolated not only from humans but also from veterinary sources⁴.

The essential oils (EO) from aromatic plants are typically produced by steam distillation processes and the chemical composition is a variable mixture of terpenoids, low molecular weight hydrocarbons, acids, alcohols, aldehydes, coumarins and other compound groups (eg. phenolic compounds)⁵. The antimicrobial properties of these natural products has been attributed mainly to terpenes and derivatives, able to interact on different target molecules and bacterial cells functions with antimicrobial mechanisms mainly of inhibition of nucleic acid synthesis, disturbance in the cytoplasmic membrane properties and energy me-
Rosmarinus officinalis L. (Labiatae) (rosemary) has medicinal uses in the prevention and/or cure of diseases, such as lack of appetite, asthma, tonsillitis, nasal obstruction and constipation and as antihypertensive, anti-inflammatory and antibacterial and their pharmacological properties such as anti-inflammatory, antibacterial, anesthetic, hypotensive, immunostimulatory and cytostatic activities were reported.

Baccharis dracunculifolia DC (Asteraceae) ("alecrim do campo") is a producer of a variety of secondary metabolites medicinally recognised as antifungal, antimicrobial and antibacterial and their pharmacological properties such as antiviral, antifungal, anesthetic, hypotensive, immunostimulatory and cytostatic activities were reported.

Vernonia polyanthes Less (Asteraceae) ("assa peixe") is used in folk medicine against rheumatism, bronchitis, coughs, flu, pneumonia, fevers, malaria, hypertension and other diseases.

Matricaria recutita L (Asteraceae) (chamomile) has been used to treat conditions such as wounds, ulcers, eczema, gout, skin irritations, neuralgia, rheumatism, pains, haemorrhoids, mastitis, rashes and conjunctivitis and its EO is rich in terpenoids, including α-bisabolol oxides and derivatives, azulenes (including chamazulene), farnesene and spathulenol.

Cinnamomum zeylanicum Blume (Lauraceae) (cinnamon) has been used throughout history against several diseases including antifungal, anti-parasitic, antibacterial and larvicidal properties and its main compound is cinnamaldehyde.

Caryophyllus aromaticus L (Myrtaceae) (clove) is used for several purposes, including oral hygiene products, antiseptic, analgesic, antifungal, anti-carcinogenic allergenic, mutagenic, insecticide and antibacterial and eugenol, main component of the EO, is responsible for their antimicrobial activities of clove and studies suggest its use as a natural food preservative.

Infusions and decoctions from Eugenia uniflora L (Myrtaceae) ("pitanga") are used in Brazil as anti-diarrheal, diuretic, anti-rheumatic, anti-fever, anti-tussive, expectorant, digestive, carminative, and controlling blood pressure, cholesterol and uric acid, and in reducing weight.

Therefore, in view of the high indications of medicinal plant uses and the possibility of EO extraction, we aimed to investigate the antimicrobial activities of essential oils from seven plants against pathogenic bacteria isolated from human clinical specimens and chemical characterisation by GC-MS of EO essential oils produced from the organs (bark, leaf and flower) from the plants included in this study.

## EXPERIMENTAL

### 2.1 Plants samples

Clove (C. aromaticus-inflorescences), cinnamon (C. zeylanicum-bark), "pitanga" (E. uniflora-leaves), rosemary (R. officinalis-leaves) and chamomile (M. recutita-flowers) samples were purchased in local supplier of Botucatu/São Paulo State, Brazil and "assa peixe" (V. polyanthes-leaves) and "alecrim do campo" (B. dracunculifolia-leaves) samples were harvested from native Botucatu area.

Plants not commercially available were identified and their respective vouchers deposited in the Herbarium Dr. Irina Delanova Gemtchujnicov of Department of Botany-IBB-UNESP-Botucatu, Brazil according to the numbers: chamomile-25794-Botu, alecrim do campo-25795-Botu, pitanga-25796-Botu and assa peixe-25797-Botu.

### 2.2 Essential oils (EO) production and chemical analysis

All EO were produced by steam distillation using Clevenger apparatus (Marconi M480) in the Department of Microbiology and Immunology/IBB-UNESP and used in the susceptibility tests without any special preparation. The EO yields and densities were determined and chemical characterisation was performed by gas chromatography-mass spectrometer (GC-MS), Shimadzu model QP5050A, according to operating conditions: CBP-5 capillary column (50m × 0.25 mm × 0.25 μm), injector temperature of 250°C, and helium (He) as a carrier gas. The energy of impact (EI) used in MS was 70 eV. The identification of the compounds in the EO were found by mass spectra analysis according to the NIST library and described in the literature and the quantification was used by area under the curve from the GC. Combinations were prepared at 1:1 ratio with the intention to also present the results of the chemical associations between EO.

### 2.3 Bacterial strains

Isolates from human specimens and standard ATCC strains were tested, and were kept frozen at −70°C (Department of Microbiology and Immunology/IBB). The strains tested were S. aureus (16 methicillin-resistant (MRSA) and 15 methicillin-sensitive (MSSA) strains), E. coli (15 strains), P. aeruginosa (15 strains) and Salmonella (15 clinical isolates of Salmonella Typhimurium and 16 isolates from food of S. Enteritidis). Reference strains (American Type Culture Collection - ATCC) were also included (S. aureus ATCC 25923, E. coli ATCC 25922, P. aeruginosa ATCC 27853 and S. Typhimurium ATCC 14028). MRSA and MSSA strains were isolated and identified from clinical specimens in a previous study according to conventional biochemical techniques. These were used for the identification and determination of resistance to oxacinil by the disk diffusion method with oxacinil (1 μg) and cefoxitin (30 μg) and detection of the mecA gene by
Polymerase Chain Reaction (PCR) to confirm the results. The tests were carried out with the consent of the Ethics in Research of Medicine College of UNESP, Botucatu (Protocol 5/2010).

2.4 Minimal Inhibitory Concentration (MIC) susceptibility assays

The susceptibility assays were performed in accordance with the Clinical and Laboratory Standards Institute by the agar dilution method. Petri plates and Mueller Hinton Agar (MHA), plus 0.5% Tween 80, were prepared in order to dilute the EO homogeneously at concentrations from 0.25 to 100 mg/mL. The 32 overnight bacterial cultures (37°C) into Brain Heart Broth (BHI) were standardized by 0.5 McFarland scale and inoculated in Petri dishes with the aid of the Steer multi-inoculator at 10^5 Colony Forming Units (CFU/mL). As a positive control, bacterial strains were inoculated on MHA plus 0.5% Tween 80. The MIC readings were made after 37°C/24 hours in the presence or absence of bacterial growth for each line set and the corresponding values of MIC90% for each group of microorganisms were calculated. All tests for MIC were performed in triplicate.

2.5 Susceptibility assays by time kill curve and synergism between EO

The bacterial susceptibility tests were also performed by time kill curve in order to check the bactericidal or bacteriostatic effect of EO according to the MIC90% found previously. Four EO were chosen according to their antibacterial activities and yield during EO extraction. Six combinations at 1/4 of the MIC90% for each EO were carried out to check the improvement of antibacterial activities against only S. aureus (ATCC 25923) and E. coli (ATCC 25922). Tubes with 20 mL of Mueller Hinton Broth (MHB) plus Tween 80 0.5% v/v and volumes of the respective EO were prepared at concentrations of MIC90% for oils alone and with 1/4 of MIC90% of oils according to the proposed combinations. As a positive control (bacterial growth), tubes with BHI at 0.5% Tween 80 were used without EO addition. Each tube was inoculated with standardised bacterial suspension at the 0.5 McFarland scale aiming to obtain a bacterial concentration of around 10^5 CFU/mL; these were incubated at 37°C.

Aliquots (0.1 mL) were taken at 0, 1.5, 3, 6, 9, 12 and 24 hours of the experiment and plating was done (Pour Plate) into MHA. After incubation of these sub-cultures (37°C/24 hours) the colonies forming units (CFU/mL) were performed using a colony counter (Phoenix) and the log CFU/mL was calculated. The assays were performed in triplicates. Generally, a three or more log10 reduction in bacterial counts in the antimicrobial assays indicates an adequate bactericidal response and the bacteriostatic effect when agent inhibits the bacterial growth.

2.6 Statistical Analysis

The SAS statistical software (version 9.0), licensed by the UNESP (2009) was used for the comparative analysis between treatments. Kruskal-Wallis analysis was used to detect difference in antibacterial potential between the EO tested, and statistical differences were considered significant when p<0.05.

3 Results and discussion

The density (mg/mL) and yield (%) of EO from the specified vegetal organs are presented in Table 1. As reported in the literature, we note that the extraction yield of EO for medicinal and aromatic plants is relatively low and the best results achieved were for C. aromaticus (0.61%) and R. officinalis (0.58%); the lowest yield was seen for V. polyanthes (0.15%).

The chemical characterisations (%) of EO alone are shown in Table 2 and 1:1 mixtures in Table 3. There are high concentrations of cinnamaldehyde in C. zeylanicum EO (86.31%), eugenol (75.85%) in C. aromaticus EO and chamazulene (31.48%) in M. recutita EO. There were no conflicting results in the literature for these plants and their main compounds found in this study. In the combination of C. aromaticus and C. zeylanicum, the proportional concentrations of the compounds eugenol and cinnamaldehyde (39.44 and 38.22%, respectively) were found compared with the concentrations in the respective EO alone.

The MIC90% values of bacterial strains are shown in Table 4. Note the differences between the concentrations of EO required to inhibit the growth of Gram-positive (S. aureus) and Gram-negative (E. coli, P. aeruginosa and Salmonella sp) bacteria, thereby confirming the greater susceptibility of Gram-positive bacteria to natural products. It was found that the C. zeylanicum and C. aromaticus EO presented the higher antibacterial activity, while the E. uniflora EO showed the lowest antimicrobial activity, except for a

Table 1 Density (mg/ml), yields (%) and selected organs from plants for essential oils production.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R. officinalis</th>
<th>B. dracunculifolia</th>
<th>V. polyanthes</th>
<th>M. recutita</th>
<th>C. zeylanicum</th>
<th>C. aromaticus</th>
<th>E. uniflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (mg/mL)</td>
<td>865</td>
<td>932</td>
<td>850</td>
<td>907</td>
<td>1035</td>
<td>1001</td>
<td>924</td>
</tr>
<tr>
<td>Yields (%)</td>
<td>0.58</td>
<td>0.31</td>
<td>0.15</td>
<td>0.17</td>
<td>0.35</td>
<td>0.61</td>
<td>0.19</td>
</tr>
<tr>
<td>Plant organs</td>
<td>leaves</td>
<td>leaves</td>
<td>leaves</td>
<td>flowers</td>
<td>bark</td>
<td>inflorescences</td>
<td>leaves</td>
</tr>
</tbody>
</table>

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slightly better effect against *S. aureus* strains.

The time kill curve results against *S. aureus* are shown in Fig. 1, with the bactericidal effect for *C. aromaticus* and *R. officinalis* EO and the bacteriostatic effect of *C. zeylanicum* and *B. dracunculifolia* EO alone. The bactericidal effect of *C. zeylanicum* and *R. officinalis* and bacteriostatic effect with *C. aromaticus* and *B. dracunculifolia* EO alone were found against *E. coli* (Fig. 2), and a synergistic bactericidal effect for the combination *C. zeylanicum* and *R. officinalis* was also found.

The extraction yields ranged from 0.15 mL/kg to 0.61 mL/kg. The yield is affected by several factors, including species and plant organs and the process employed for EO extraction, physiological variations inherent of the plant (phase development, pollination cycle, seasonal and plant stress conditions).
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Table 4  MIC<sub>90%</sub> (mg/mL) values against bacterial strains from clinical specimens and food samples.

<table>
<thead>
<tr>
<th>Bacteria (n)</th>
<th>Essential oils</th>
<th>R. officinalis</th>
<th>B. dracunculifolia</th>
<th>V. polyanthes</th>
<th>M. recutita</th>
<th>C. zeylanicum</th>
<th>C. aromaticus</th>
<th>E. uniflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (32)</td>
<td>8.60 ± 0.85</td>
<td>7.10 ± 1.75</td>
<td>26.20 ± 3.45</td>
<td>26.50 ± 2.63</td>
<td>0.25 ± 0.04</td>
<td>2.70 ± 0.20</td>
<td>55.60 ± 2.20</td>
<td></td>
</tr>
<tr>
<td>MRSA* (16)</td>
<td>8.60 ± 0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.70 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.20 ± 3.63&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.80 ± 3.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.25 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.70 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.00 ± 3.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MSSA** (15)</td>
<td>8.60 ± 0.90&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.40 ± 1.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.30 ± 3.05&lt;sup&gt;x&lt;/sup&gt;</td>
<td>26.60 ± 4.77&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.25 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.80 ± 3.80&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Salmonella (32)</td>
<td>79.44 ± 3.56</td>
<td>56.00 ± 3.83</td>
<td>25.50 ± 2.55</td>
<td>54.40 ± 3.55</td>
<td>0.25 ± 0.03</td>
<td>2.40 ± 0.27</td>
<td>92.40 ± 6.20</td>
<td></td>
</tr>
<tr>
<td>S. Enteritidis (16)</td>
<td>79.39 ± 3.83&lt;sup&gt;d&lt;/sup&gt;</td>
<td>56.00 ± 2.85&lt;sup&gt;h&lt;/sup&gt;</td>
<td>25.50 ± 2.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.40 ± 1.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.25 ± 0.02&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>2.40 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.40 ± 6.90&lt;sup&gt;ae&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>S. Typhimurium (15) (infection)</td>
<td>79.48 ± 2.58&lt;sup&gt;ae&lt;/sup&gt;</td>
<td>56.00 ± 1.96&lt;sup&gt;ad&lt;/sup&gt;</td>
<td>25.50 ± 1.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.40 ± 2.70&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>0.25 ± 0.02&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>2.50 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.40 ± 8.20&lt;sup&gt;if&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>E. coli (16)</td>
<td>79.35 ± 2.98&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>56.00 ± 4.81&lt;sup&gt;ae&lt;/sup&gt;</td>
<td>51.00 ± 4.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.40 ± 5.80&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.25 ± 0.02&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>3.00 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.30 ± 7.40&lt;sup&gt;gh&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa (16)</td>
<td>79.91 ± 3.40&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>56.00 ± 4.55&lt;sup&gt;eh&lt;/sup&gt;</td>
<td>51.00 ± 4.95&lt;sup&gt;ce&lt;/sup&gt;</td>
<td>54.40 ± 4.65&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.80 ± 0.01&lt;sup&gt;aa&lt;/sup&gt;</td>
<td>3.00 ± 0.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>92.40 ± 8.00&lt;sup&gt;eh&lt;/sup&gt;</td>
<td></td>
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</table>

*MRSA (methicillin-resistant S. aureus), **MSSA (methicillin-sensitive S. aureus)

Different letters in columns (capital letters) and line (lowercase) indicate a significant difference (p<0.05).

Fig. 1  Time kill curve against S. aureus (ATCC 25923) with essential oils alone at MIC<sub>90%</sub> and combinations at ¼ of MIC<sub>90%</sub> values.

...tions), environmental and geographic conditions (climate, air pollution, soil characteristics), and the genetic characteristics of cultivars. As for yield, which is the quantitative aspect, the EO composition (qualitative aspect) can also be influenced by these parameters. As the EO antimicrobial activities depend on their chemical composition, we compared the main compounds of EO samples achieved by GC-MS analysis with previous studies, as well as the intrinsic resistance of microorganisms, revealed by MIC<sub>90%</sub>, to discuss the results.

GC-MS analysis of R. officinalis EO was found 1,8-cineole (26.54%), α-pinene (20.14%), camphor (12.88%) and camphene (11.38%) as the main compounds. According to the EO chemical compositions shows differences according to extraction method and reported that R. officinalis essential oil extracted by hydrodistillation presented the major compounds the verbenone (17.43%), camphor (16.57%), 1,8-cineole (11.91%) and α-pinene (11.47%). The antibacterial activity of this EO was evaluated and MIC values of 3.75 mg/mL against S. aureus (ATCC 6538) and 7.5 mg/mL against E. coli (ATCC 8739) were found. Despite being one of the same plant species, the analysis of EO extracted in our laboratory showed a yield of 0.58% and different compositions, i.e. camphor

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Fig 2 Time kill curve against *E. coli* (ATCC 25922) with essential oils alone at MIC<sub>90</sub> and combinations at ¼ of MIC<sub>90</sub> values.

(27.51%) limonene (21.01%) and myrcene (11.19%) as the main compounds. By comparing the MIC values against *S. aureus* and *E. coli*, strongly different results (8.60 and 79.35 mg/mL respectively) were also seen. Thus, the EO analysis and susceptibility profile of bacterial strains is an important tool for interpretations because normally the clinical isolates exhibit superior resistance to antimicrobials after the frequent exposure in hospital environments.

Hydrodistillation, although the most frequent process, may cause changes in the chemical composition of the EO produced because high temperatures can sometimes cause the loss of the more volatile compounds. *B. dracunculifolia* EO obtained hydrodistillation presented nerolidol (22.16%) and β-pinene (12.17%) and a yield of 0.6% as well as the presence of nerolidol (33.51%) and spathulenol (16.24%) as major compounds to *B. dracunculifolia* EO. While setting a lower yield (0.31%), a similar chemical characterisation of *B. dracunculifolia* EO (nerolidol 25.84% and 13.14% of spathulenol) was found in this study (Table 2). The inhibitory activity of *B. dracunculifolia* against *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 using the disk diffusion method was reported. Likewise, *B. dracunculifolia* EO showed antibacterial activity, especially against *S. aureus* strains (Table 4), by agar dilution method.

While *V. polyanthes* EO is poorly characterised, there are reports on the potential of this plant, mainly using extracts aimed anti-ulcer, anti-fungal and anti-leishmanic activities. The germacrene D (27.79%), α-caryophyllene (16.02%) and germacrene B (15.01%) were main compounds of its EO and the statistical analyses of *V. polyanthes* EO antibacterial activities show a slight effectiveness against Gram-positive bacteria and *Salmonella* from both sources (infections and food) (Table 4).

*M. recutita* has been used over several generations in different ways and purposes and over 120 compounds have been identified in the EO. In 13 samples from Europe, the existence of 39 compounds was reported, with the main being bisabolol oxide A (3.1 to 56.0%), α-bisabolol (0.1 to 44.2%), and bisabolol oxide B (3.9-27.2%) and chamazulene (0.7 to 15.3%) and caryophyllene (13.59%) being the most significant. Antimicrobial activity described for *M. recutita* EO varies with susceptibility assays and microorganisms evaluated, and some studies have described conflicting results. According susceptibility assays results from *M. recutita* EO against streptococcal the MIC ranged from 0.1 to 7.0 mg/mL. The MIC values for *M. recutita* EO reported here range from 25.80 to 54.40 mg/mL, which are higher values than in the literature.

*Cinnamomum* comprises about 250 species, with *C. zeylanicum* being the most important; the compound found in the EO prepared with leaves is eugenol, and in
bark is cinnamaldehyde\textsuperscript{38}. \textit{C. zeylanicum} EO was one of the most efficient, with MIC\textsubscript{90} of 0.25 mg/mL against all bacteria tested, except against \textit{P. aeruginosa} strains (0.80 mg/mL), although this is a satisfactory result. The GC-MS analysis shows high amounts of cinnamaldehyde (86.31\%) and cinnamyl acetate (4.83\%) (Table 2).

With the higher extraction yield (0.61 mL/kg), the \textit{C. aromaticus} EO GC-MS analysis found indicated eugenol (75.85\%) and ethyl eugenol (16.38\%) as major compounds and the antibacterial activity was seen at concentrations ranging from 2.4 to 3.0 mg/mL. Although without chemical characterisation of EO tested, Silva \textit{et al.}\textsuperscript{39} also reported that the EO of \textit{C. aromaticus} was the most effective against \textit{S. aureus} and \textit{E. coli}, with the values of MIC\textsubscript{90} of 0.095\% and 0.895\% v/v respectively. Zago \textit{et al.}\textsuperscript{39} reported the antimicrobial activity of \textit{C. aromaticus} EO (0.095 \%/v/v) and a greater activity of \textit{C. zeylanicum} EO against strains of \textit{S. aureus} (0.047 \%/v/v). Thus, these results were around those found in this study and verified for most EO from these two plants (Table 4).

In the EO from fresh leaves of \textit{E. uniflora} was reported the presence of atractilona (26.78\%), curzereno (17.96\%) and germacrone B (9.31\%), and the results showed again that antimicrobial activity was varied against fungi and Gram-positive bacteria strains (MIC 0.11 to 7.5 mg/mL respectively) without any effect against Gram-negative strains\textsuperscript{40}. The MIC\textsubscript{90} values ranged from 50.80 to 92.40 mg/mL, which was higher than those observed in other studies; one explanation may be related to the source of bacterial strains (e.g. isolated from human clinical specimens and hospital settings) \textsuperscript{41}.

The chemical analysis of \textit{E. uniflora} EO showed that the main compounds were selina 1,3,7(11) trien-8-one (30.10\%), selena epoxide 1,3,7(11) trien-8-one (21.89\%) and β-caryophyllene (6.51\%) (Table 2). There was seasonal variation in the presence of compounds compared the EO chemical analysis from leaves collected in the dry and wet season \textsuperscript{42}. Thus, we conclude that variations in chemical composition may justifiably differences in the antimicrobial potential of the same plant species.

All EO showed antimicrobial activities, thus demonstrating the potential of using these plants as antibacterial agents. Depending on the plant species and the bacterial strains tested, these activities can be classified as bacteriostatic or bactericidal, this being the purpose of the tests of time kill curve employing the respective values of MIC 90\%. Thus, considering the results of these tests on \textit{S. aureus} and \textit{E. coli} ATCC, these were different, because \textit{C. aromaticus} EO was bactericidal against \textit{S. aureus} and bacteriostatic on \textit{E. coli}. The opposite was found for \textit{C. zeylanicum} EO, which was bactericidal for \textit{E. coli} and bacteriostatic against \textit{S. aureus} growth.

With regard to synergism between natural products, Probst \textit{et al.}\textsuperscript{43} reported synergism between essential oils of ginger (\textit{Zingiber officinale}) and mint (\textit{Mentha piperita}) and also \textit{C. zeylanicum} and \textit{Z. officinalis}; however, this study did not report the chemical composition of the EO tested. There was synergism for all combinations and a significant result of bactericidal effect by \textit{C. aromaticus} and \textit{R. officinalis} against \textit{S. aureus} and \textit{C. zeylanicum} and \textit{R. officinalis} against \textit{E. coli} strains. Thus, it is noteworthy that besides the synergism revealed by \textit{R. officinalis} with two combinations, this plant showed also excellent productivity in essential oil (0.58\%) surpassed most plants tested. This is certainly an interesting agronomic viewpoint, and especially economic, when the goal is the production of this essential oil.

There are some possible explanations of the differences in the antibacterial activity of oils: structural features of Gram-positive and Gram-negative bacteria; the chemical composition and concentrations of each EO tested; variations in the chemical composition of the EO according to the plant growth conditions; the developed conditions of the plant and finally the EO extraction process. Thus, the chemical composition of EO also interferes with its potential antimicrobial activity, which is associated with the content of terpenoids in the oil \textsuperscript{44}. According to the results from chemical analysis, it was shown that the major isolated compounds are mostly terpenes or derivatives from this class (Table 2).

Typically, Gram-positive bacteria are more sensitive when exposed to EO \textit{in vitro} tests than Gram-negative bacteria \textsuperscript{45}. The peculiar Gram-negative characteristics, presenting an outer membrane composed of lipopolysaccharide (LPS) in the bacterial wall, provide greater resistance to prevent the spread and accumulation of EO in the bacterial cell.

Although there was no significant difference, we observed that MSSA strains were more resistant or showed the same sensibility levels as the MRSA strains for most EO. Brady \textit{et al.}\textsuperscript{46} reported similar results when they evaluated the susceptibility of MRSA and MSSA strains to tea tree EO (\textit{Melaleuca alternifolia}) and reported MIC between 0.25 to 2.0 \%/v/v and 0.5%-2.0\% v/v, respectively, although statistically significant difference was not found for both \textit{S. aureus} strains. The EO \textit{M. piperita} has demonstrated antibacterial activity at concentrations of 0.064 to 0.128 mg/mL against MSSA and from 0.064 to 0.256 mg/mL against MRSA strains, although no significant differences were found between the bacterial types tested\textsuperscript{46}. These results suggest a good perspective in therapeutic alternatives for the treatment of MRSA infections that are precisely the most difficult to treat with conventional antibiotics.

In evaluating the sensitivity profile of strains of \textit{Salmonella} spp. isolated from food and human infections, there were no significant differences (Table 4). The antibacterial activity of EO extracted from seven species of \textit{Citrus}...
against 11 strains of Salmonella spp. also revealed that MIC values were not statistically significant. In the literature, other studies evaluating the potential anti-Salmonella of EO report no difference in inhibitory concentrations against Salmonella Enteritidis and S. Typhimurium, although some authors have noted differences in the MIC for strains of these serovars.

Although recent studies have shown the chemical composition of the EO used in microbiological assays, we think that it is not possible to relate the antibacterial activity of certain compounds with EO accurately. There are also studies that have reported the bioactivity of some EO as products of the interaction of various compounds, and not just because of the action of the isolated compounds.

4 Conclusions

Thus, we conclude that all of the EO tested showed antibacterial action and that this activity was higher or lower depending on the composition of each oil and bacteria tested. Further studies are needed in order to investigate the antimicrobial mechanism of these compounds found in these EO well as increase the antibacterial activity of these EO.

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